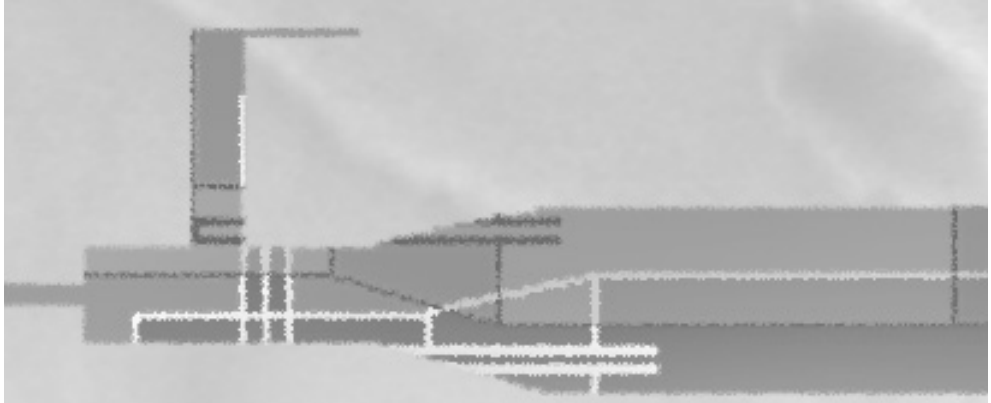
Design and development of a miniaturised flow-through measuring device for the in vivo monitoring of glucose and lactate

Rhemrev-Boom, Maria Martha



Chapter 3

Biosensor device and ultrafiltration sampling for continuous in vivo monitoring of glucose

Summary

A biosensor was tested for its applicability for the *in vivo* monitoring of glucose and sampling by ultrafiltration at submicroliter flow rate. The biosensor was integrated in a Flow Injection Analysis system and the performance characteristics of the biosensor were determined. In addition, the first results obtained with a miniaturised portable sensor device for the *in vivo* monitoring of glucose are demonstrated.

3.1 Introduction

It is well known that intravascular sensors have the disadvantage of rapid induction of blood clotting, and that most sensors suffer from serious drawback sensor performance due to fouling and contamination of the surface during operation[1]. In order to avoid this problem, microdialysis was proposed as an interface between the body and the sensor[2-4]. An alternative for *in vivo* sampling is ultrafiltration[5]. In both cases, the sample is free of large proteins and analysis can be performed nearly without further purification. An advantage of ultrafiltration over microdialysis is that the analyte of interest is nearly quantitatively recovered without dilution of the matrix sampled. However, to obtain a representative sample during monitoring, low volume samples are required. Therefore, the use of a small disposable syringe for pulse-free ultrafiltration at submicroliter flow rates have been reported[6,7]. Sampling was performed up to 24 hours and in this case on-line analysis was performed electrochemically using a bi-enzyme reactor in a flow injection system[8]. To circumvent the use of expensive equipment and to miniaturise the analytical system to improve the patient's mobility, the application of a biosensor seems justified. Besides being small, robust and easy to handle, these biosensors must be biocompatible and reliable for measurements in the complex matrix of body fluids. For instance for glucose and lactate measurements, amperometric biosensors with immobilised enzymes for the conversion of the analyte into electrochemically detectable products, are frequently applied. Since several years, various authors have described the production of biosensors utilising various methods of enzyme immobilisation on electrodes in order to improve the selectivity of the biosensor and to overcome interference from electroactive species. The use of a biosensor for off-line discontinuous monitoring is mostly described, but for continuous *in vivo* monitoring of patients little or no data have been reported. Therefore, in our laboratory we started to test several commercially available biosensors. In this article we describe the

experiments carried out and the results obtained with one commercially available biosensor. For the purpose of *in vivo* on-line monitoring of blood during ultrafiltration at low flow rate, the biosensor was tested for its sensitivity, stability and selectivity. To enhance the stability of the sensor and the linear dynamic range of the assay, the biosensor was integrated into a miniaturised Flow Injection Analysis (FIA) system.

Our ultimate goal is to define a miniaturised portable biosensor device for on-line continuous *in vivo* monitoring. Sampling should preferably be performed by means of ultrafiltration at submicroliter flow rate. Although many attempts have been reported to miniaturise biosensor devices, no hardware is yet available to perform *in vivo* sampling by ultrafiltration at 100 nl.min^{-1} and direct analysis by means of a portable biosensor device. Besides the need for a pulse free pump capable of delivering reproducibly low flow rates (100 nl.min^{-1}), extremely low internal volumes are required for these low volumes of sample. By using the disposable syringe as mentioned before[6,7], the first results of a home-made biosensor device which is capable of sampling through ultrafiltration at low flow rate and on-line analysis by means of a portable biosensor are demonstrated in this article.

3.2 Materials and Methods

3.2.1 Materials

D+-Glucose for standard solutions was obtained from Sigma Chemical Concentration. (St. Louis, MO). All other chemicals were of pro-analysis quality and purchased from Merck (Darmstadt, Germany). Double quartz distilled water was used for all aqueous solutions containing 0.1% (by volume) Kathon CG (Rhom and Haas, Croydon, UK) to inhibit bacterial growth. The composition of the carrier solution was a Dulbecco's phosphate-buffered saline (PBS) (mmol.l^{-1}): NaCl (136.9), KCl (2.7), KH_2PO_4 (1.5), $CaCl_2$ (0.9), $MgCl_2$ (0.5), and Na_2HPO_4 (8.1). Prior to analysis, helium was purged through the carrier solution to remove air. The stock glucose solution (50 mmol.l^{-1}) was prepared by dissolving glucose in PBS and allowed to stand for 24 hours. The standard solutions were prepared from this stock solution by diluting with PBS.

3.2.2 Methods

Sampling by ultrafiltration was performed by a probe (fibers of an artificial kidney, AN69HF,

acrylonitrile and sodium methallyl sulphonate copolymer, Filtral 16, Hospal Ind., Meyzieu, France, 340 µm o.d., 240 µm i.d.) of 2 cm with a hand-made spring inside (stainless steel wire, D = 60 µm, 12 axial length windings per cm, Vogelsang, Hagen, Germany). One end of this probe was connected via a 20-30 cm long fused silica tube (i.d. 50 µm, o.d. 150 µm, Polymicron Technologies inc., Phoenix, Arizona) with a model Marathon Autosampler (Spark Holland, Emmen, the Netherlands). The probe was filled with water and the other end of the probe was closed with cyano-acrylic glue (Henkel, Nieuwegein, the Netherlands). Ultrafiltration at a flow rate of 100 nl.min-1 was carried out by the underpressure of a model 22 syringe pump (Harvard Apparatus, Kent, GB). The syringe was directly connected to the autosampler. The valve of the autosampler was automatically switched on a time-basis (load/inject 60/60 seconds). The autosampler was equipped with a 20 µL loop, which was partially filled (100 nl) with ultrafiltrate. A model LKB 2150 HPLC pump (Pharmacy Brim, Sweden) was used to pump the carrier solution at a flow rate of 0.5 ml.min-1 via the autosampler through a specially designed flow-through cell. The GOD sensor (SensLab, Leipzig, Germany) was inserted in the flow-through cell and connected to a home-made potentiostate. The potential applied was held at +250 mV vs. Ag/AgCl. The signal output was recorded with a model BD112 flatbed recorder (Kipp & Zonen, Delft, the Netherlands).

3.3 Results and discussion

After testing several commercially available biosensors, promising results were obtained with the biosensors from SensLab. However, to be able to test this biosensor for our purpose, we had to construct a specific flow-through cell. The constructed flow-through cell as well as the instrumental set-up is demonstrated in figure 1. By using the instrumental set-

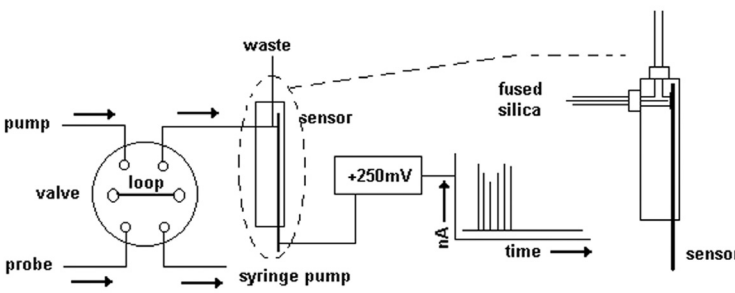


Figure 1:
Instrumental set-up for the determination of glucose.

up and the conditions mentioned in 3.2.2., the biosensor device was tested for its performance characteristics. The repeatability of the assay was determined by analysing in sixfold standard solutions of glucose of between 0.5 - 15 mmol of glucose. The repeatability, expressed as the relative standard deviation in the peak height, was found to be 1- 2 %. By analysing standard solutions of glucose in PBS, the assay was found to be at least linear up to 15 mmol (correlation coefficient of > 0.99). An example of a calibration curve is shown in figure 2. The selectivity of the biosensor was tested by analysing accordingly a standard solution of 0.15 mmol of ascorbic acid in PBS. If a diagnostic concentration of 0.04 mmol of ascorbic acid is assumed, the contribution of ascorbic acid to the peak height for glucose was calculated to be approximately 3%. The accuracy of the biosensor was tested by analysing a pooled blood sample by the hexokinase reference method (GlucoTrend, Boehringer Mannheim, Germany) as well as with the method demonstrated here. Prior to analysis, a calibration plot was calculated after analysing standard solutions of glucose in PBS. The calculated content of glucose in the sample was found to be 6.57 mmol, whereas with the reference method a glucose content of 6.67 mmol was found. With respect to the stability of the GOD sensor, standard glucose solutions in PBS as well as a pooled serum sample were monitored for up to 12 hours and no significant decline in the signal for glucose was noticed. Equilibrium after placing the biosensor in the flow-through cell was reached within several minutes, and, although a slight decrease in the signal for glucose was seen, one sensor could be used for up to 14 days. A typical chart record of monitoring by means of the method described here is presented in figure 3. From this figure the drift in the peak heights due to the pulse of the model 22 Harvard syringe pump can be easily recognised.

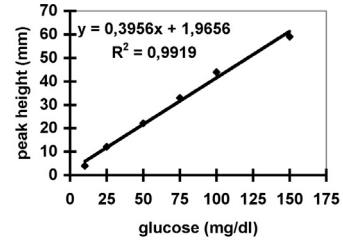


Figure 2:
Calibration curve for the determination of glucose by FIA in combination with the tested biosensor.

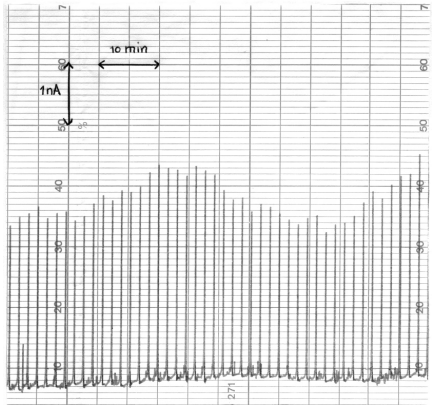


Figure 3:
Typical chart record of the determination of glucose in vivo by means of ultrafiltration and FIA.

In addition to the promising results obtained with the biosensor mentioned above, further attempts have been made regarding miniaturisation of our disposable sensor device. Therefore, the pulse-free disposable syringe pump, as reported earlier[6,7], was tested for its performance characteristics. During several days we followed the performance of the syringe pump (1.2 mL Monovette, Sarstedt, Nümbrecht, Germany, equipped with a restriction fused silica tube, 15 µm i.d., 150 µm o.d., 4 cm length, glued into the syringe) by sampling water at a flow rate of 300 nl.min^{-1} through ultrafiltration and weighing with a microbalance (Sartorius, Nieuwegein, the Netherlands) every five minutes the amount of ultrafiltrate sampled. The data were directly recorded and are presented in figure 4. The 24 hours of the recorded data presented here are typical for the performance of the syringe pump; based on these results it can be concluded that the performance characteristics of the pump are adequate for our purpose. Next, a home-made sensor was constructed by us as presented in figure 5. Ultrafiltration was carried out at a flow rate of 200 nl.min^{-1} by the underpressure of the pulse-free disposable syringe as mentioned above. Sample was introduced every minute for 30 seconds through a 25 µm i.d. fused silica tube via a home-made valve. Instead of using a diffusion controlled membrane or biocatalytic elimination as reported by many authors, in order to improve the selectivity of the biosensor we used rhodium to catalyse the electroreduction of hydrogen peroxide. In comparison with earlier results[9], electroreduction of the biochemical reaction product hydrogen peroxide could be

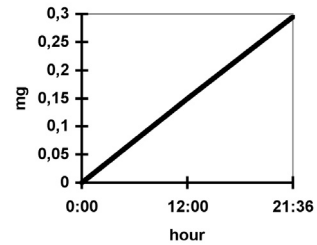


Figure 4:
Performance of the pulse-free disposable syringe pump at 300 nl.min^{-1}.

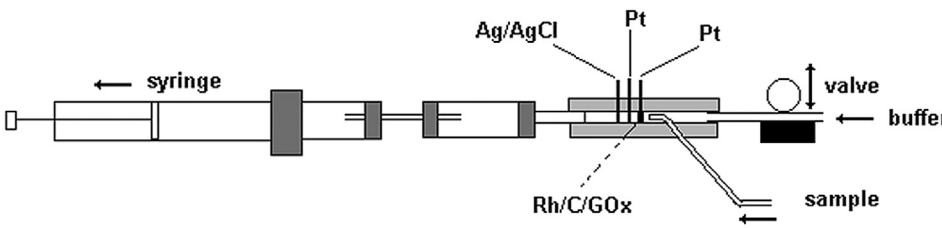


Figure 5:
Schematic construction of the home-made biosensor.

established at -150 mV. At this potential no interference of the signal by ascorbic acid was noticed by us. So far promising results have been obtained by this home-made biosensor device. In the near future, however, more work has to be done regarding the sensitivity and the dimensions of the biosensor device before we can start the experiments for *in vivo* monitoring of patients. In order to achieve in an efficient manner our ultimate goal, we will also continue with the testing of the biosensors mentioned here as well as other available biosensors.

3.4 Comments on biomedical applications

In this article we demonstrated the possibilities for the on-line and continuous *in vivo* monitoring of glucose after sampling by ultrafiltration at very low flow rates. On-line analysis can be performed with FIA or a miniaturised biosensor device. Based on the performance characteristics found for the biosensor tested here, we will proceed with some relevant biomedical applications, such as the monitoring of glucose in men and rats after subcutaneous ultrafiltration and glucose in rat after intravenous ultrafiltration. Our first measurements with a lactate sensor show that monitoring of both glucose and lactate at the same time belongs to the possibilities.

3.5 References

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